Focus on kidney cancer

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Introduction

Annually in the United States, kidney cancer affects nearly 32,000 individuals and is responsible for over 12,000 deaths. It is estimated that 200,000 individuals are living with kidney cancer in the U.S. If kidney cancer is detected and treated early and the tumor is localized to the kidney, patients can have a good disease-specific survival rate (95% at 5 years). However, when patients present with advanced disease, they have only an 18% two-year survival rate (Linehan et al., 2003). Over the past 20 years, there have been major advances in our understanding of the epidemiology, pathology, genetics, and treatment of renal cell carcinoma.

Epidemiology

Since the mid 1970s, kidney cancer has increased at a rate of about 2.5%/year in the United States. The highest rates of increase were observed in African Americans. Multiple case control studies have been performed to identify risk factors for the development of renal carcinoma. Cigarette smoking, hypertension/antihypertensive medications, obesity, and family history (see below) are the major risk factors that have been identified for the development of renal carcinoma. The population attributable risk for hypertension was 21%, for excess weight was 21%, and for was smoking 18%. At least 40% of the renal cancer cases were not explained by the risk factors studied (McLaughlin and Lipworth, 2000).

Studies of dominantly inherited, epithelial forms of renal carcinoma

In a small proportion of cases, the predisposition to renal carcinoma occurs as a dominantly inherited, autosomal trait. Rare familial renal cancers have been studied intensively for clues to the pathogenesis of the more common, sporadic forms of renal carcinoma. To date, four types of inherited epithelial forms of renal carcinoma have been delineated clinically, and genes responsible for them have been characterized by positional cloning. Each disease has distinct clinical characteristics, and is caused by mutation in a distinct gene. There are family pedigrees that suggest that other types of dominantly inherited kidney tumors remain to be identified (Teh et al., 1997).

von Hippel Lindau: Clear cell renal carcinoma

Individuals affected with von Hippel Lindau (VHL) are at risk for the development of tumors in a number of organs, including the kidneys, pancreas, adrenal glands, brain, spine, eye, and inner ear. VHL patients are at risk for the development of bilateral, multifocal, early onset kidney tumors and cysts. The tumors are uniformly of clear cell histologic type, and the walls of the renal cysts have clear cell linings. Allelic loss of VHL has been detected in these renal cysts, and they are thought to be preneoplastic (Lubensky et al., 1996). Often, renal tumors grow from the cells that line the renal cysts. It has been estimated that VHL patients are at risk for the development of up to 600 clear cell renal neoplasms and 1100 cysts per kidney (Walther et al., 1995). These tumors are malignant, can metastasize, and may appear as early as the second decade of the patient's life. Historically, 35%–45% of VHL patients have died of metastatic renal carcinoma.

Identification of the VHL gene, germline mutations, and genotype phenotype correlations

The *VHL* gene was identified by positional cloning in 1993 (Latif et al., 1993). Complete and partial *VHL* gene deletions, frameshift, and missense mutations were found to lead to the development of VHL (Zbar et al., 1996). Von Hippel-Lindau disease is classified into distinct clinical subtypes based on the presence or absence of pheochromocytoma, and the presence or absence of renal carcinoma (Zbar et al., 1996).

Constitutional chromosome 3 translocation: A unique model of inherited VHL gene-associated clear cell RCC

In 1979, Cohen et al. reported a family carrying a constitutional balanced translocation (t[3;8] [p 14;q24]) who had a high risk of developing bilateral, multifocal clear cell renal carcinoma (Cohen et al., 1979). In the tumor tissue from the patients in the 3;8 translocation family, the translocated chromosome 3 was lost, and in 2 of the 4 t(3;8)-associated renal tumors, somatic *VHL* mutations were found (Schmidt et al., 1995). Koolen et al. reported another chromosome 3 translocation (t[2;3] [q34;q21]) family with multiple members with multifocal clear cell kidney cancer (Koolen et al., 1998). Analysis of the *VHL* gene in the kidney tumors revealed somatic mutations in four of five tumors tested (Bodmer et al., 1998). In an analysis of 10 chromosome 3 translocation families, Geurts van Kessel et al. found a substantial increase in risk of renal cell carcinoma in carriers of reciprocal chromosome 3 translocations (Geurts van Kessel et al., 1999).

VHL gene pathway: Opportunity for disease-specific approaches to therapy

When the *VHL* gene was identified, it was a unique gene with unknown function. Since that time, considerable progress has been made to elucidate its function (Jaakkola et al., 2001; Maxwell et al., 1999, 2001; Kaelin, 2003; Ivan et al., 2001; Iliopoulos et al., 1995). The VHL protein forms a complex with elongins C and B, Cul-2, NEDD8, and Rbx1 to target the α subunit of the hypoxia-inducible factors (HIF1α and HIF2α) for ubiquitin mediated degradation (Duan et al., 1995; Kibel et al., 1995; Pause et al., 1997; Kamura et al., 1999; Stickle et al., 2004; Kaelin, 2002; Pugh and Ratcliffe, 2003a). This is a hypoxia-regulated process; under normoxic conditions, the HIF complex is degraded, whereas in hypoxia, HIF1 and HIF2 are not degraded, and they accumulate (Kaelin, 2003; Pugh and Ratcliffe, 2003b). The interaction between the pVHL E3 ligase complex and the HIF α subunits is preceded by enzymatic prolyl hydroxylation of HIF, which is the oxygen-sensitive step in the degra-

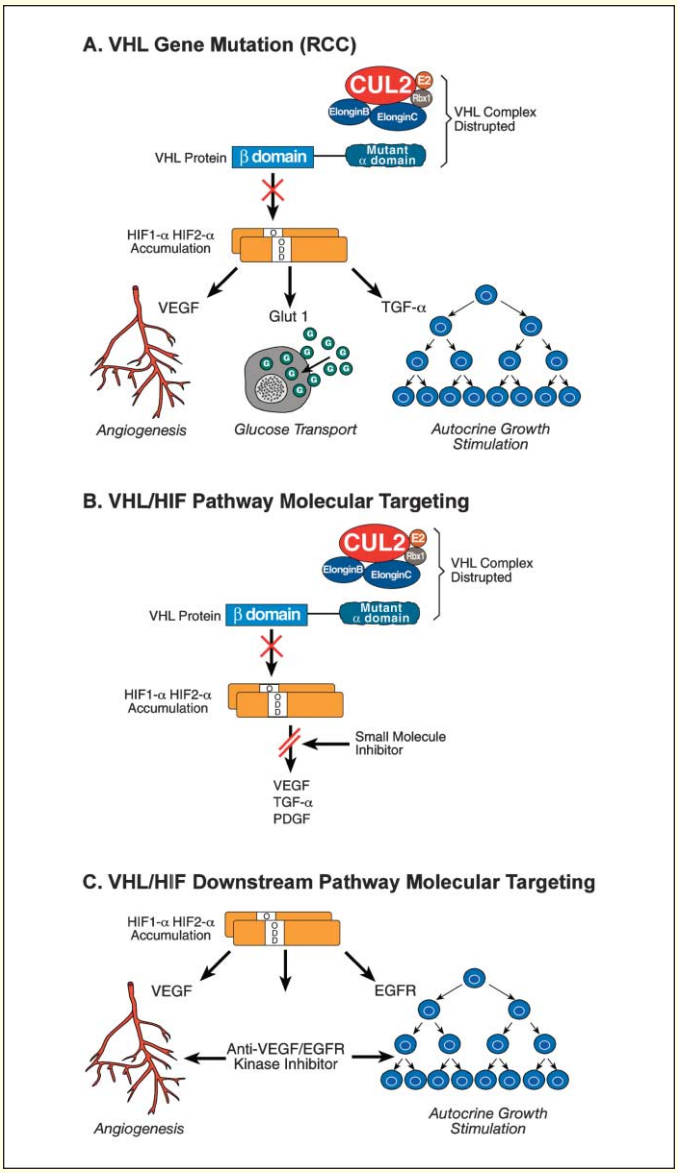

Figure 1. Kidney cancer is not a single disease, it is made up of a number of different types of cancer that occur in the kidney

These different types of kidney cancer are characterized by different histologies, have different clinical courses, respond differently to therapy, and are associated with alteration of different genes. Modified from Linehan et al. (2003).

dation pathway (Jaakkola et al., 2001; Ivan et al., 2001; Yu et al., 2001). Two interfaces have been described for *VHL*: the α domain interacts with elonginC-elonginB (Stebbins et al., 1999), and the β domain interacts with HIF (Ohh et al., 2000). Mutations in the α domain prevent assembly of the VHL-elonginC-elonginB-Rbx1 complex; mutations in the β domain inhibit binding and degradation of HIF (Stebbins et al., 1999; Ohh et al., 2000). Mutations of the *VHL* gene in either the α or β domain result in accumulation of HIF, even in normoxic conditions. Increased accumulation of HIF results in increased transcription of a number of genes, including VEGF, EGFR, TGFα, Glut 1, and PDGF (Figure 1).

Role of VHL in tumorigenesis

Studies have shown that competing the VHL complex from binding to HIF proteins results in tumorigenesis (Maranchie et al., 2002), and that mutations in the oxygen-dependent domain (the VHL binding site) of HIF2α, but not HIF1α, result in tumorigenesis in xenograft models (Maranchie et al., 2002; Kondo et al., 2002). Kondo et al. and Zimmer et al. showed that inhibition of HIF2α is sufficient to suppress pVHL-deficient tumor growth (Kondo et al., 2002, 2003; Zimmer et al., 2004). These studies establish that HIF2α is a critical component to tumorigenesis in clear cell renal carcinoma.

Hereditary papillary renal carcinoma: Type 1 papillary RCC

Hereditary papillary renal carcinoma (HPRC) is an autosomal dominant hereditary cancer syndrome in which affected individuals are at risk for the development of bilateral, multifocal, type 1 papillary renal carcinoma (Zbar et al., 1994). It is estimated that HPRC patients are at risk for the development of up to 3,400 tumors per kidney (Ornstein et al., 2000). In contrast to the other forms of epithelial renal carcinoma, in HPRC, the disease manifestations are confined to the kidney.

The HPRC gene was shown to be the proto-oncogene, *c-Met*, located on the long arm of chromosome 7 (Schmidt et al., 1997). Met is a cell surface receptor tyrosine kinase for the ligand hepatocyte growth factor. Activating mutations in the tyrosine kinase domain of *Met* were found in the germline of affected individuals in HPRC kindreds. These germline mutations in the *MET* gene were located in codons homologous to those mutated in tyrosine kinases that cause other human diseases (Schmidt et al., 1998). *Met* mutations have also been found in a subset of tumors from patients with sporadic, type 1 papillary renal carcinoma (Schmidt et al., 1999).

Birt-Hogg-Dubé: Chromophobe and oncocytic renal cell carcinoma

Birt-Hogg-Dubé (BHD) is a hereditary cancer syndrome in which affected individuals are at risk for the development of cutaneous fibrofolliculoma, pulmonary cysts, and pneumothorax and renal tumors (Zbar et al., 2002). 82% of BHD patients have pulmonary cysts, and 22% have a history of pneumothorax. Kidney tumors occur in 15%–25% of BHD patients and can be bilateral and multifocal. In the NCI series, 33% of BHD kidney tumors are chromophobe renal carcinoma, 50% are oncocytic renal carcinoma, 9% are clear cell, and 6% oncocytoma.

The *BHD* gene was located on the short arm of chromosome 17, and shown to be a novel gene without structural similarities to known genes (Schmidt et al., 2001; Nickerson et al., 2002). Germline mutations of the *BHD* gene were identified in ~80% of BHD kindreds. The *BHD* gene contains a homonucleotide tract, a set of 8 cytosines in exon 10, that is a hot spot for germline mutations. About 50% of BHD families studied have a germline insertion or deletion of a single cytosine residue at the cytosine homonucleotide tract. Virtually all germline mutations in the *BHD* gene are predicted to truncate the BHD protein, folliculin.

Hereditary leiomyomatosis renal cell carcinoma

Hereditary leiomyomatosis renal cell carcinoma (HLRCC) is a hereditary cancer syndrome in which affected individuals are at risk for the development of cutaneous and uterine leiomyoma and kidney cancer (Launonen et al., 2001). Toro et al. reported that a high percentage of women with cutaneous leiomyomas

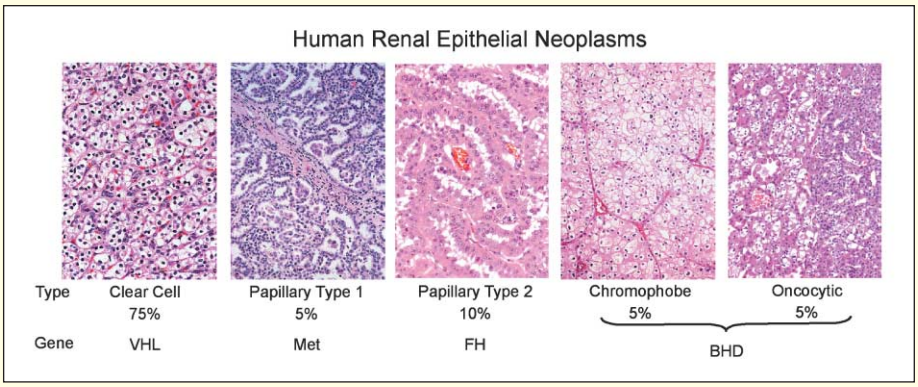

Figure 2. Molecular targeting of the VHL pathway in clear cell renal carcinoma

Mutation of the VHL gene in clear cell kidney cancer results in increased accumulation of HIF and the resulting increase in transcription of downstream targets such as VEGFR, EGFR, and TGFα (A). Mutation of the VHL gene in the α domain (shown here) inhibits binding to elongin C and formation of the VHL complex (Stebbins et al., 1999). Mutation in other parts of the gene, such as the β domain, prevents binding to and ubiquitin mediated degradation of HIF (Ohh et al., 2000). Potential disease-specific therapeutic approaches include agents which block the function of HIF (B), VEGFR, or EGFR (C). Modified from Linehan et al. (2002, 2003).

(98%) have uterine leiomyomas (mean age of diagnosis: 30 years) and that 91% of the women with cutaneous and uterine leiomyomas had undergone either a hysterectomy or uterine myomectomy (Toro et al., 2003). The renal tumors that develop in patients affected with HLRCC have a characteristic histologic appearance, similar to type 2 papillary renal carcinomas and collecting duct carcinomas of the kidney.

The gene for HLRCC is the Krebs cycle enzyme fumarate hydratase (*FH*) (TMLC, 2002), which has the characteristics of a tumor suppressor gene. Inactivating mutations have been found in the germline, and LOH has been detected in HLRCC-associated kidney tumors (Kiuru et al., 2001). Studies are currently underway to determine how inactivation of the Krebs cycle enzyme fumarate hydratase leads to the development of kidney cancer.

Principles derived from the study of inherited renal carcinomas

The studies of inherited renal carcinomas emphasize the genetic and biologic diversity of inherited epithelial tumors of the kidney. Of the four renal carcinoma genes identified, three (*VHL*, *BHD*, and *FH*) are tumor suppressor genes, and one (*Met*) is a proto-oncogene. The *MET* gene encodes a transmembrane receptor tyrosine kinase, the fumarate hydratase gene carcinoma gene encodes a Krebs cycle enzyme, and the *VHL* gene regulates the degradation of a transcription factor (HIF). Although the clinical and pathologic phenotypes associated with germline alterations of these genes are distinct, recent studies suggest that the downstream pathways may overlap. For example, Pennacchietti et al. discovered that hypoxia induces *c-Met* (Pennacchietti et al., 2003). Eng et al. have raised the provocative notion of a renal tumorigenesis pathway in which severe energy deficits from impaired mitochondrial function and creation of large amounts of oxygen free radicals are important for promoting cellular growth and the development of renal neoplasia (Eng et al., 2003).

Epidemiologic and family studies suggest the presence of renal carcinoma susceptibility genes

Epidemiology studies of renal carcinoma have suggested that a family history of renal carcinoma is a risk factor for the disease. The relative risk to a sibling of a patient affected with renal cancer is estimated at 2.5. This observation was reproducible in a number of epidemiologic studies. Of particular interest are the studies of renal carcinoma in the Sweden Family-Cancer Database, which includes all Swedes born since 1931 and their biological parents. Hemminki analyzed this database followed up to year 2000 and observed that risks between siblings were particularly high for renal cancer. Relative risk to siblings greater than the relative risk in parent/child pairs suggested to Hemminki the contribution of a recessive gene(s) to the development of sporadic renal carcinoma (Hemminki and Li, 2004a, 2004b).

Investigators in Iceland performed a comprehensive study of all patients in Iceland who had developed kidney cancer between 1955 and 1999 (1078 RCC cases). Utilizing an extensive computerized database containing geneologic information on over 600,000 individuals in Iceland over the past 11 centuries, they were able to conduct a unique analysis to determine the familial nature of "sporadic" kidney cancer. The results of this study revealed that nearly 60% of patients in Iceland during this time period had a first or a second degree relative with kidney cancer (Gudbjartsson et al., 2002).

There have been reports of non-VHL, non-HPRC kidney cancer families (Teh et al., 1997; Woodward et al., 2000). Czene and Hemminki identified 71 families in Sweden in which both a parent and an offspring had kidney cancer (Czene and Hemminki, 2002). In population-based studies, kidney cancers have been associated with other types of cancers, including prostate cancer (Benichou et al., 1998).

Sporadic kidney cancer

VHL gene mutation and LOH is found in a high percentage of tumors from patients with noninherited clear cell kidney cancer, suggesting a tumor suppressor role for the *VHL* gene (Gnarra et al., 1994). When the *VHL* gene was placed in $VHL^{-/-}$ kidney cancer cells, tumorigenicity in xenograft models was suppressed (Kaelin and Maher, 1998), further supporting a tumor suppressor role for VHL (Figure 2).

Although the *Met* gene has been found to be mutated in a subset of sporadic type 1 papillary renal carcinomas (Schmidt et al., 1999), the genes responsible for the majority of sporadic type 1 papillary, type 2 papillary, and chromophobe renal carcinoma have yet to be determined. A type of papillary renal carcinoma associated with translocations involving the X chromosome (Xp11.2) has been described (Shipley et al., 1995) in which there is a fusion to the *TFE3* transcription factor (PRCC-TFE3, NONO-TFE3, ASPL-TFE3, PSF-TFE3) (Weterman et al., 1996; Clark et al., 1997). These studies suggest that a dysregulated fusion protein may be central to the neoplastic process in this malignancy. This tumor, which often occurs in children, has also been associated with abnormality of the *TFE3*-related family member, *TFEB*, which was recently

cloned in a pediatric papillary renal carcinoma (Davis et al., 2003).

Conventional and experimental treatment of sporadic kidney cancer

Laparoscopic nephrectomy is the current therapy for patients with tumors greater than 4 centimeters, regardless of histologic type; partial nephrectomy is recommended for patients with smaller tumors. Treatment with Interleukin-2 (IL-2), the current FDA-approved agent for patients with advanced renal carcinoma, is associated with a complete response in 10% and partial response in 11% of patients. IL-2 therapy is usually only recommended for patients with clear cell renal carcinoma (Yang et al., 2003b).

Experimental treatment for advanced renal carcinoma includes stem cell transfusions from HLA-compatible siblings following immunoablative chemotherapy (Childs et al., 2000), treatment with anti-VEGF antibody (Yang et al., 2003a), and vaccination with autologous tumor cells. Recent meeting reports indicate encouraging results from agents with c-Raf and VEGFR inhibitory properties (M.J. Ratain et al., 2004, J. Clin. Oncol., abstract), VEGFR inhibitors (SU11248) (R.J. Motzer et al., 2004, J. Clin. Oncol., abstract), and combinations of agents which target both the VEGFR and EGFR pathways (J.D. Hainsworth et al., 2004, J. Clin. Oncol., abstract).

Another potential therapeutic approach involves interactions of the molecular chaperone HSP90 and its client proteins. HSP90 stabilizes a number of client proteins, including HIF1α, EGFR (the receptor for TGFα), and c-Met. Geldanamycin is an HSP90 antagonist which has been shown to promote efficient ubiquitination and proteosome-mediated degradation of HIF1α in RCC in both normoxia and hypoxia (Isaacs et al., 2002). Clinical trials are underway to evaluate the effect of geldanamycin analogs 17-allylamino-17-demethoxygeldanamycin (17AAG) and 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG) in patients with kidney cancer.

A number of approaches are under study to block the signal transduction pathway in c-Met associated tumors, such as blockade of kinase activation with small molecule inhibitors of ATP binding, blocking the interaction between activated c-Met and its downstream signaling molecules, and blockade of the HGF-Met interactions (Linehan et al., 2003; Kong-Beltran et al., 2004; Zhang et al., 2004; Michieli et al., 2004).

Targeting single gene pathways

Detailed study of kidneys in VHL and HPRC patients reveals that patients affected with VHL are at risk for the development of up to 600 tumors per kidney and that affected HPRC patients are at risk for the development of up to 3,400 tumors per kidney (Walther et al., 1995; Ornstein et al., 2000). BHD patients are at risk to develop hundreds of microscopic oncocytic tumors (Pavlovich et al., 2002). These findings plus the studies showing suppression of tumorigenesis of VHL$^{-/-}$ RCC cell lines by (1) replacement of the *VHL* gene (Iliopoulos et al., 1995) or (2) inhibition of HIF2α (Kondo et al., 2003; Zimmer et al., 2004) suggest that abnormality of a single gene may be critical for the development of these tumors and provide rationale for strategies targeting a single gene pathway.

Summary

Kidney cancer is not a single cancer; it is made up of a number of different types of cancer, with different histologies and different clinical courses, responding to different forms of therapy, and each associated with alteration of a different gene. Understanding the genetic basis of kidney cancer provides a unique opportunity for the development of disease-specific forms of therapy.

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